COMPONENTS OF OIL DERIVED FROM LIQUEFACTION OF HYDROCARBON-RICH MICROALGAE

Seiichi INOUE, Shigeki SAWAYAMA and Tomoko OGI
National Institute for Resources and Environment, 16-3 Onogawa, Tsukuba, Ibaraki 305, Japan

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ABSTRACT

Botryococcus braunii is a colonial green microalga that produces and accumulates oily hydrocarbons called botryococcenes(C30-36). Liquefaction was applied to *B. braunii* for recovery of hydrocarbons. The liquefied oil was obtained with yield of 64% at 300°C. The oil was fractionated into three fractions by silica gel column chromatography and analyzed to determine its composition. The yields of three fractions were 5% of low molecular weight hydrocarbons, formed by degradation of botryococcenes, 27.2% of botryococcenes and 22.2% of polar substances, produced from organic materials other than hydrocarbons through liquefaction. Further analysis using GC-MS identified some components of the oil. Main components of low molecular hydrocarbons and polar substances were C17-22 hydrocarbons and C14-20 fatty acids, respectively.

INTRODUCTION

The use of biomass for energy is largely motivated from the standpoint of global environmental issues. Biomass-derived fuels would reduce greenhouse gas concentrations to the extent that they could replace fossil fuels currently being used. *Botryococcus braunii* is a unique colonial green microalga that produces and accumulates oily hydrocarbons, called botryococcenes (C30-36), with a dry weight range of 17-86%.[1] These hydrocarbons can be upgraded to transport fuels by hydrocracking[2] or catalytic cracking[3] after being recovered from the algal cells.

On a laboratory scale, the hydrocarbons of algal cells are separated by extraction with organic solvent after their freeze-drying and sonicating. However, this method is not suitable for large scale separations due to the high cost. Therefore, an effective method is needed for separating hydrocarbons from algal cells. We have proposed a new method for separating hydrocarbons from algal cells by direct liquefaction. Applying this process to *B.braunii*, a greater amount of oil than that obtained from *B.braunii* was achieved with yields of 57-64% at 300°C compared to our previous work.[4] Liquefaction could be an effective method for converting the algal cells of *B.braunii* into oil.

The analysis of the oil is important for providing preliminary information about the conversion of organic materials and for the recovery of hydrocarbons produced from the algal cells. In this paper, we analyzed the components of the liquefied oil and determined the suitable reaction conditions needed for the recovery of botryococcenes from *B.braunii*.

EXPERIMENTAL

Algal strain and botryococcenes extraction

The Berkeley strain of *B.braunii* was used for the liquefaction. This strain produces mainly $C_{34}H_{58}$.[5] The algal cells of *B.braunii* were filtered using a 20 μm nylon mesh and then freeze-dried. The algal cells (943 g) were ultrasonically extracted with 50 ml of hexane for 30 min. It gave an oil as hexane extracts (50% of dry cell weight). The extract was chromatographed on a silica gel column with hexane as the eluent in order to obtain Botryococcenes (36% of dry cell weight).

Liquefaction

Liquefaction of *B.braunii* was performed in a 300 ml autoclave at 200, 300 or 340°C, with or without Na_2CO_3 as the catalyst. Liquefied oil was extracted from the reaction mixture with CH_2Cl_2. Details of the liquefaction procedure were reported in a previous study.[4]

Fractionation

The liquefied oil was separated into three fractions. The procedure for fractionation is provided in Fig. 1. The liquefied oil was chromatographed on a silica gel column with 200 ml hexane and 200 ml diethylether. The first 100 ml of the hexane eluate gave F1. The next 100 ml of the hexane eluate was F2, and the 200 ml of the diethylether eluate was F3.

Analysis

The mean molecular weight of the respective fractions was measured using the 117 Molecular Weight Apparatus (Corona, Japan). Elemental composition of the respective fractions was analyzed using a 2400 CHN Elemental Analyzer (Perkin-Elmer, USA). The GC analysis was carried out using a capillary column (DB-1, 30 m x 0.25 mm, Hewlett-Packard, USA). The initial oven temperature was held at 100°C for 10 min, then heated to 220°C at 3 °C min^{-1}. The temperatures of the injector and FID were 220°C and 240°C, respectively. The GC-MS (DB-1, 30 m x 0.25 mm, Hewlett-Packard, USA) analysis was carried out under the same condition.

RESULTS AND DISCUSSION

The properties of F1, F2 and F3 and of botryococcenes extracted from the algal cells are shown in Table 1. The mean molecular weight of F2 was in the range of 438-572, and that of

botryococcenes in the algal cells was 472. The elemental composition of F2 was in good agreement with that of botryococcenes. In addition, the GC retention time of F2 coincided well with the retention time of the botryococcenes. From these results, F2 was identified as the botryococcenes. Elemental analysis showed that F1 was hydrocarbons and the mean molecular weight of F1 was in the range of 197-281. These results suggest that F1 is some kind of degraded product of the botryococcenes. F3 contained oxygenated compounds. Since F3 contained oxygen compounds, they may be produced from organic materials except for the hydrocarbons through liquefaction. From these results, we could confirm that F1, F2 and F3 were low molecular weight hydrocarbons, botryococcenes and polar substances, respectively.

Figure 2 shows the yield of each fraction of the liquefied oil and hexane extracts from the algal cells. The recovery (75%) of the botryococcenes in the liquefied oil was obtained when the liquefaction was carried out at 300°C in the presence of a catalyst (5% sodium carbonate). The yields of the three fractions based on an organic basis were 5% low molecular weight hydrocarbons, 27.2% botryococcenes, and 22.2% polar substances.

The recovery of the botryococcenes was improved with the use of a catalyst at the reaction temperatures of 200 and 300°C (Fig. 2). The quantitative difference in the recovery of the botryococcenes between 200°C and 300°C with a catalyst was very small, but recovery at 340°C decreased. The reaction temperature of 200°C is a preferable reaction temperature from the standpoint of energy use. The liquefaction process can be used for recovery of the botryococcenes as well as the conversion of the algal cells into oil. The total recovery of the fractions (F1, F2 and F3) was in the range of 70-88 wt% for the liquefied oils. The liquefied oil of *B. braunii* had a small amount (1%) of nitrogen, but the fractions (F1, F2 and F3) from the silica gel column contained no nitrogen. The loss is most likely due to the strong adsorption of highly polar components in the liquefied oil onto the silica gel.

Further analysis using GC-MS identified some components of the liquefied oil obtained at 300°C with a catalyst. Table 2 shows the main components of low molecular hydrocarbons and polar substances. The main components of low molecular hydrocarbons were C17-22 hydrocarbons. It is thought that these compounds were formed by degradation of botryococcenes in the algal cells. On the other hand, the main components of the polar substances were C14-20 fatty acids. Except for these compounds, many compounds were identified. From the standpoint of the reaction mechanism during the liquefaction of *B.braunii*, it is very interesting that a methoxyl compound was identified. Ether compounds were found in some strains of *B.braunii*.[6,7] The methoxyl compound may be produced during the liquefaction. Phenol and pyrrolidine compounds, which are absent in the cell, were also identified. They are formed by the degradation and combination of the constituents, such as proteins, in the algal cell.

CONCLUSION

The oil derived from the liquefaction of *B.braunii* consisted of three fractions: low molecular hydrocarbons, botryococcenes and polar substances. From an analysis using GC-MS of the oil obtained at 300°C with a catalyst, the main components of the low molecular hydrocarbons and polar substances were C17-22 hydrocarbons and C14-20 fatty acids, respectively. The recovery of botryococcenes was 75%. The recovery of the botryococcenes was improved using a catalyst at reaction temperatures of 200 and 300°C.

REFERENCES

1. T.G. Tornabene, Microorganisms as hydrocarbon producers *Experientia* **38**, 43-46 (1982).
2. L.W. Hillen, G. Pollard, L.V. Wake and N. White, Hydrocracking of oils of *Botryococcus braunii* to transport fuels. *Biotechnol. Bioeng.* **XXIV**, 193-205 (1982).
3. H. Kitazato, S. Asaoka and H. Iwamoto, Catalytic cracking of hydrocarbons from microalgae. *Sekiyu Gakkaishi* **32**(1), 28-34 (1989).
4. Y. Dote, S. Sawayama, S. Inoue, T. Minowa and S. Yokoyama, Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel* **73**, 1855-1857 (1994)
5. J. R. Maxell, A. G. Douglas, G. Eglinton, and A. McCormick, The botryococcenes-hydrocarbons of novel structure from the alga Botryococcus braunii, Kützing. *Photochemistry* **7**, 2157-2171 (1968).
6. P. Metzger and E. Casadevall, Botryococcoid ethers, ether lipids from *Botryococcus braunii*. *Photochemistry* **30**, 1439 (1991).
7. P. Metzger and E. Casadevall, Ether lipids from *Botryococcus braunii* and their biosynthesis. *Photochemistry* **31**, 2341 (1992).

Table 1 Properties of the fractionated oil and botryococccenes

	Mean molecular weight	Elemental composition			GC retention time
		C(%)	H(%)	O(%)	(sec)
Fraction 1	197-281	84.5-85.5	14.6-15.5	0.0	-3540
Fraction 2	438-572	85.0-86.5	13.2-14.0	0.0-1.0	4140-4380
Fraction 3	867-2209	73.3-77.3	12.2-13.3	10.1-14.3	4680-
Botryococcenes	472	85.7	14.3	0.0	4140-4380

Table 2 Components of liquefied oil

Low molecular weight hydrocarbon
5-Octadecene
Heptadecane
$C_{19}H_{38}$
$C_{20}H_{40}$
Octadecane
Tetramethyl hexadecene
Docosane
Polar substances
Mesityl oxide
Diacetone alcohol
Phenol
Methyl phenol
Ethyl phenol
2,6-Bis(1,1-dimethylethyl)-4-ethyl phenol
Tetradecanoic acid
Trimethyl pentadecanone
Methyl tetradecanoic acid
Hexadecanoic acid
9-Octadecenoic acid
Octadecanoic acid
Eicosanoic acid
1-(7-Methyl-1-oxopentadecyl)pyrrolidine

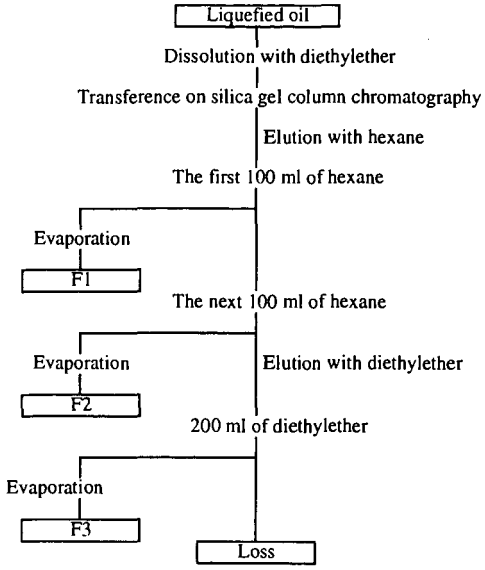

Fig. 1. Procedure for fractionation of liquefied oil

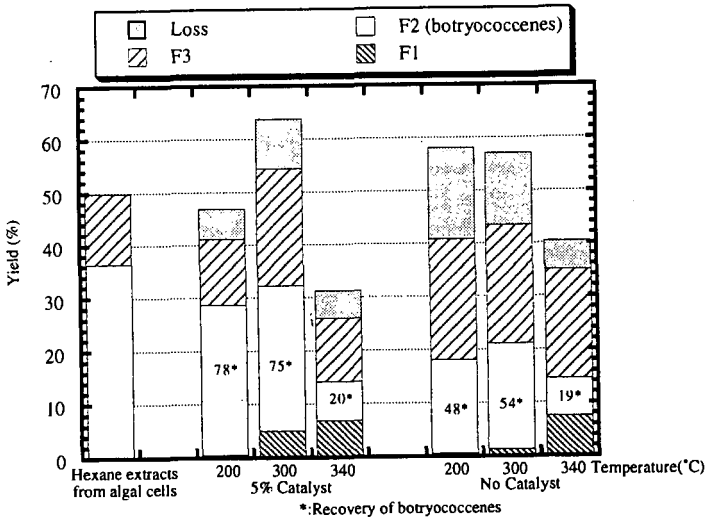

Fig. 2 Yield of each fraction of liquefied oil and hexane extracts from algal cells.